IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 032026/0476D

In re patent application of FREY, *et al.*

Application No. Group Art Unit:

Filed: May 1, 2001 Examiner:

For: **DNA MOLECULES ENCODING BACTERIAL LYSINE 2,3-AMINOMUTASE**

PRELIMINARY AMENDMENT

Box PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is a Preliminary Amendment filed with a Divisional Application for the above-captioned application. It is requested that this preliminary amendment be entered after the application has been accorded a filing date and been assigned a serial number.

AMENDMENTS

Please delete the title and insert therefore "METHOD FOR PRODUCING L-β-LYSINE"

Please cancel claims 1-28 and 33-35.

Please amend the claims as follows:

29. (Amended) A method of producing L-β-lysine, comprising:

(a) culturing a host cell comprising an expression vector that encodes lysine 2,3-aminomutase in the presence of L-lysine, wherein the cultured host cell expresses lysine 2,3-aminomutase, and

(b) isolating L-β-lysine from the cultured host cells.

30. (Amended) A method of producing L-β-lysine, comprising:

(a) incubating L-lysine in a solution containing purified lysine 2,3-aminomutase, said solution containing all cofactors required for lysine 2,3-aminomutase activity; and

(b) isolating L-β-lysine from the incubation solution.

31. (Amended) The method of claim 30, wherein the lysine 2,3-aminomutase has an amino acid sequence selected from the group consisting of (i) SEQ ID NO:4, (ii) SEQ ID NO:6, (iii) SEQ ID NO:8, (iv) SEQ ID NO:10, (v) SEQ ID NO:12, (vi) SEQ ID NO:14, (vii) SEQ ID NO:16, (viii) SEQ ID NO:2 and (ix) a conservative amino acid variant of any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, or 16.

36. (New) The method of claim 29 wherein the vector that encodes lysine 2,3-aminomutase has a nucleic acid sequence selected from the group consisting of (i) SEQ ID NO: 1; (ii) (ii) SEQ ID NO: 3, (iii) SEQ ID NO: 5, (iv) SEQ ID NO: 7, (v) SEQ ID NO: 9 , (vi) SEQ ID NO:11, (vii) SEQ ID NO:13, and (viii) SEQ ID NO:15.

37. (New) The method of claim 29 wherein the isolated L-β-lysine is enantomerically pure.

38. (New) The method of claim 30 wherein the isolated L-β-lysine is enantomerically pure.

39. (New) The method of claim 30 wherein the cofactors required for lysine 2,3-aminomutase activity comprise:

(i) at least one of ferrous sulfate or ferric ammonium sulfate;

(ii) pyridoxal phosphate;

(iii) at least one of dehydrolipoic acid, glutathione or dithiothreitol;

(iv) S-adenosylmethionine; and

(v) sodium dithionite.

40. (New) A method of producing L-β-lysine, comprising:

(a) immobilizing lysine 2,3-aminomutase on a suitable support;

(b) activating the lysine 2,3-aminomutase with cofactors required for lysine 2,3-aminomutase activity; and

(c) contacting L-lysine with the immobilized lysine 2,3-aminomutase to produce L-β-lysine.

41. (New) The method of claim 37 wherein the L-lysine is contacted with the immobilized lysine 2,3-aminomutase for a sufficient amount of time to produce enantomerically pure L-β-lysine.

42. (New) The method of claim 37 further comprising separating the L-β-lysine from the L-lysine.

43. (New) The method of claim 42 wherein the separation of the L-β-lysine from the L-lysine is achieved using high performance chromatography.

44. (New) The method of claim 37 wherein the process is a continuous process.

45. (New) The method of claim 37 wherein the cofactors required for lysine 2,3-aminomutase activity comprise:

(i) at least one of ferrous sulfate or ferric ammonium sulfate;

(ii) pyridoxal phosphate;

(iii) at least one of dehydrolipoic acid, glutathione or dithiothreitol;

(iv) S-adenosylmethionine; and

(v) sodium dithionite.

46. (New) The method of claim 37, wherein the lysine 2,3-aminomutase has an amino acid sequence selected from the group consisting of (i) SEQ ID NO:2, (ii) SEQ ID NO:4, (iii) SEQ ID NO:6, (iv) SEQ ID NO:8, (v) SEQ ID NO:10, (vi) SEQ ID NO:12, (vii) SEQ ID NO:14, (viii) SEQ ID NO:16 and (ix) a conservative amino acid variant of any of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, or 16.

REMARKS

After entry of this amendment, Claims 29-32 and 36-46 are pending in the application, Claims 1-28 and 33-35 have been canceled. Claim 29 has been amended to be independent and include all of the elements of the claims previously recited therein, claim 30 has simply been amended to make explicit what was inherent in the claim, claim 31 has been amended to include an additional member in the Markush group thereby broadening the scope of the claim, claim 32 is retained in its original form and claims 36-44 have been added to more thoroughly claim the invention. Accordingly, none of the amendments decrease the scope of any equivalents of the claims under the doctrine of equivalents. A marked-up copy of claims 29-31 is attached hereto wherein the changes to the claims are marked as follows: additions are underlined and deletions are in brackets.

The present amendment adds no new matter and is otherwise proper. Entry of the amendment in its entirety is respectfully requested. Support for these claims is found throughout the application as filed, including, but not limited to:

claim 29: claims 1, 26, 27 and 29 as originally filed;

claim 30: page 26, line 29 through page 27, line 3 and claim 30 as originally filed;

claim 31: page 5, lines 18-19 and claim 31 as originally filed;

claim 36: claims 4, 7, 10, 13, 16, 19, 22 and 25 as originally filed;

claim 37: page 26, lines 25-26 and page 27, lines 10-19;

claim 38: page 26, lines 28-29 and page 27, lines 10-19;

claim 39: page 26, line 29 through page 27, line 3;

claim 40: page 27, lines 4-9;

claim 41: page 27, lines 4-18;

claim 42: page 27, lines 14-18;

claim 43: page 27, lines 15-18;

claim 44: page 27, lines 4-5;

claim 45: page 26, line 29 through page 27, line 3; and

claim 46: page 5, lines 18-19 and claim 31 as originally filed.

Applicants believe that the application is in condition for allowance and favorable action thereon is respectfully solicited.

Respectfully Submitted,

Mark A. Kassel, Reg. No. 38,200
Attorney for Applicants
Foley & Lardner
150 East Gilman Street
Post Office Box 1497
Madison, Wisconsin 53701-1497
(608) 258-4272

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(a) culturing a host cell comprising an expression vector that encodes lysine 2,3-aminomutase in the presence of L-lysine, wherein the cultured host cell expresses lysine 2,3-aminomutase, and

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